REMARKS

The present invention relates to regulatory T cells (Treg cells) and methods of long-term, culture-expanding, activating and using same in immunotherapy and for the suppression of autoimmune responses.

By way of the present Amendment, claims 1 and 3 have been amended. Claim 36 has been added to indicate that the isolated cells are cultured in the presence of a $CD4^+$ feeder layer or $CD4^+$ feeder layer conditioned medium. Support for this amendment is found through out the specification (e.g., See Example 8). No new matter has been added by way of these amendments.

Claim Objections

The Examiner has objected to claims 1 and 3 for matters of form. Accordingly, claim 1 has been amended to delete "GMP-approved methods"; claim 3 has been amended to recite "said".

Response to Rejections Under 35 U.S.C. 112, Second Paragraph

Claims 1-5, 7-11, and 28-35 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for reciting the phrases "GMP-approved methods", "comprises a high level of stringency", and "wherein isolation step further comprises substantially enhancing $CD4^+CD25^{bright}$ cells in said isolated population, while substantially depleting $CD25^{dim}$ cells in said isolated population". The Examiner contends that the metes and bounds of these phrases are not clear and that the skilled artisan would not know what would meet the limitations of the claimed method.

Applicants have amended claim 1 to remove the recitation of " GMP-approved methods". Thus, the rejection of the claim 1 with respect to this phrase is now moot.

With respect to the phrases "comprises a high level of stringency" and "wherein isolation step further comprises substantially enhancing $CD4^+CD25^{bright}$ cells in said isolated population, while substantially depleting $CD25^{dim}$ cells in said isolated population," Applicants respectfully submit that these phrases comply with the standard set forth under 35 U.S.C. § 112, second paragraph for the following reasons.

It is settled law that the "patent law allows the inventor to be his own lexicographer." *Chicago Steel Foundry Co. v. Burnside Steel Foundry Co.*, 132 F.2d 812 (7th Cir. 1943). *See also* MPEP § 2173.01. This is because "[t]he dictionary does not always keep abreast of the inventor. It cannot. Things are not made for the sake of words, but words for things." *Autogiro Co. v. U.S.*, 155 USPQ 697 (Ct. Cls. 1967). Further, applicant is entitled to have the claims construed in connection with the other parts of the application. *See Autogiro Co. v. U.S.*, 155 USPQ 697 (Ct. Cls. 1967). Therefore, Applicants are entitled to define terms to describe their invention and the claims must be interpreted in light of the other parts of the application including the disclosure in the specification and the definitions provided therein.

Applicants respectfully submit that the specification as filed makes clear the meaning of "comprises a high level of stringency" and "wherein isolation step further comprises substantially enhancing $CD4^{+}CD25^{bright}$ cells in said isolated population, while substantially depleting $CD25^{dim}$ cells in said isolated population," and therefore one skilled in the art would have understood, based upon the disclosure provided in the specification as filed, the meaning of these phrases.

The present invention is based on the discovery that the most stringently purified $CD4^{+}CD25^{+}$ cells form the best suppressor cell line precursors. Contaminating $CD25^{-}$dim cells in $CD25^{+}$ fractions can grow faster and overgrow the $CD25^{+}$ bright cells, and thereby preclude the full manifestation of suppressor cell function. Thus, the present invention relates to a method of isolating $CD25^{+}$ in a stringent purification system that preferably includes at least two cycles of selection and extensive washing (e.g., double column purification procedure). The specification indicates that the high level of stringency is preferred to optimize purity of $CD25^{+}$ cells (e.g., $CD25^{+}$ bright cells), even at the cost of a lower cell yield.

The specification describes that the double column purification procedure is useful in view of the fact that it is advantageous to isolate the $CD25^{bright}$ subset of $CD4^{+}CD25^{+}$ cells in order to detect suppressor activity. This is because it was observed that contamination of $CD25^{dim}$ cells in $CD25^{+}$ fractions grew faster and can overgrow the $CD25^{bright}$ cells, and thereby preclude the full manifestation of suppressor cell function (*See, e.g.*, Example 8). It was observed that $CD25^{dim}$ cells exhibited a lower suppressive activity than $CD25^{bright}$ cells (*See, e.g.*, paragraph 24, page 8). Thus, the double column purification procedure provides a high level of stringency to isolate $CD25^{bright}$ cells over $CD25^{dim}$ cells.

Based on the teachings of the specification, a skilled artisan would not be confused as to the meaning of an isolation step comprising "a high level of stringency" nor "substantially enhancing $CD4^+CD25^{bright}$ cells in said isolated population, while substantially depleting $CD25^{dim}$ cells in said isolated population," because the artisan would recognize that the claimed double column magnetic antibody cell sorting (MACS) purification procedure provides the isolation of an enhanced population of $CD4^+CD25^{bright}$ cells.

These phrases are not indefinite. Accordingly, Applicants respectfully request that the rejection of claims 1-5, 7-11, and 28-35 under 35 U.S.C. § 112, second paragraph, as being indefinite be reconsidered and withdrawn.

Rejection of claims 1-5, 7-11, and 28-35 under 35 USC §112, 1st paragraph - enablement

The Examiner has rejected claims 1-5, 7-11, and 28-35 under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement standard. The Examiner contends that the specification does not reasonably provide enablement for contacting $CD4^+CD25^+$ Treg cells with immobilized anti-CD3 antibody and immobilized anti-CD28 antibody at a predetermined ratio, wherein the ratio of the amount of anti-CD3 antibody to anti-CD28 antibody is less than 1 to produce therapeutic human Treg cells with enhanced suppressor activity. The Examiner cites Chen et al., (2003, Cytokine Growth Factor Rev. 14: 85-89) and Baecher-Allan (2001, J. Immunol. 167: 1245-1253) to demonstrate that the state of the art is unpredictable.

As an initial matter, Applicants do not understand the timing of the Examiner's rejection to the subject matter relating to culturing the cells with immobilized anti-CD3 antibody and immobilized anti-CD28 antibody at a predetermined ratio, wherein the ratio of the amount of anti-CD3 antibody to anti-CD28 antibody is less than 1. Claims 26-29 were added by way of Amendment dated January 18, 2007. Claims 27 and 28 are directed to microbeads coated with antibodies to CD3 and CD28 at a ratio of a higher amount of CD28 to CD3 and CD3 to CD28 is at least 1:5, respectively. In response to the Amendment dated January 18, 2007, the Examiner issued a Final Office Action dated April 10, 2007, wherein the only rejection to claims 27 and 28 was that they were indefinite under 35 U.S.C. §112, second paragraph, insofar as they depended from a rejected independent claim. Accordingly, Applicants amended claim 1 to at least incorporate the subject matter of claim 27 by way of Amendment dated April 8, 2008. Thus, the present rejection raised by the Examiner as to a predetermined ratio, wherein the ratio of the

amount of anti-CD3 antibody to anti-CD28 antibody is less than 1 is repugnant to the principles for compact prosecution set for in MPEP §2106. This is because the Examiner is now rejecting the subject matter previously encompassed by claim 27.

With respect to the teachings of Baecher-Allan, Applicants submit that this reference is inapplicable for the following reasons. Baecher-Allan does not use anti-CD3 antibody and anti-CD28 antibody as encompassed in the claims. Specifically, Baecher-Allan uses soluble anti-CD28 antibody in the experiments summarized in Figure 3. However, the pending claims relate to contacting $CD4^+CD25^+$ Treg cells with immobilized anti-CD3 antibody and immobilized anti-CD28 antibody. Immobilized anti-CD28 antibody is different from soluble anti-CD28 antibody. Thus, the teachings of Baecher-Allan cannot be relied upon to demonstrate that the claims lack enablement.

In addition, the present invention is based on the discovery that the most stringently purified $CD4^+CD25^+$ cells form the best suppressor cell line precursors. Contaminating $CD25^{dim}$ cells in $CD25^+$ fractions can grow faster and overgrow the $CD25^{bright}$ cells, and thereby preclude the full manifestation of suppressor cell function. Thus, the present invention relates to a method of isolating $CD25^+$ in a stringent purification system that preferably includes at least two cycles of selection and extensive washing (e.g., double column purification procedure). The specification indicates that the high level of stringency is preferred to optimize purity of $CD25^+$ cells (e.g., $CD25^+$ bright cells), even at the cost of a lower cell yield. However, in order to have a desirable number of these cells for therapeutic purposes, the inventors sought to find a method to produce sufficient number of these cells to permit safe and effective therapeutic use in human patients.

Thus, the inventors developed methods for improved cell isolation and culturing. It was observed that the more stringently the $CD4^+CD25^+$ cells were purified, the less well they grew in culture, even with stimulation with anti-CD3/CD28 beads and IL-2. However, after trying various accessory cell populations, irradiated $CD4^+$ T cells (used as "feeder cells") were found to give the best results for facilitating growth of the isolated $CD25^+$ bright cells. It was also observed that conditioned media (supernatant from anti-CD3/anti-CD28 stimulated $CD4^+$ T cells) greatly facilitated suppressor cell growth, even more so than that which resulted from IL-2 supplementation.

Accordingly, a preferred embodiment of the present invention encompasses isolating a population of $CD4^+CD25^{bright}$ cells using a highly stringent purification system (e.g., double column purification procedure) and culture expanding the $CD4^+CD25^{bright}$ using anti-CD3/28 mAb coated beads in combination with IL-2 and/or irradiated feeder cells to induce both (i) robust expansion by >100-fold and (ii) an increase in suppressor cell activity. (See paragraph [0071] of U.S. Patent Application Pub. No. 20050196386). Claim 36 has been added to claim this embodiment of the invention.

The Examiner also contends that claims 4 and 34 lack enablement because using less volume of beads compared to manufacture's recommendation would lead to incomplete collection of $CD4^+CD25^{bright}$ cells rather than enhance the isolation of $CD4^+CD25^{bright}$ cells (See Baecher-Allan et al., 2001, J. Immunol. 167: 1245-1253). Applicants disagree with the Examiner partly because Example 8 demonstrates that Applicants' successful reduced to practice the claimed invention as encompassed in claims 4 and 34. As set forth in at least Example 8, $CD25^+$ bright cells were isolated by positive selection from PBMC with directly conjugated anti-CD25 magnetic microbeads (211 per 10^7 cells) (Miltenyi Biotec, Auburn, Calif.), and purified over an LS^+ column. Cells were then applied to a second magnetic column, washed, and re-eluted. After the double column procedure, cells were routinely >93% pure (for CD25) by FACS analysis.

Thus, the present invention is partly related to the discovery that using lower titers of anti-CD25 magnetic microbeads and re-purifying the cells over a second column and culturing the cells according to the invention greatly facilitated the generation of Treg cell lines with potent suppressive capabilities. By performing more stringent purification strategies and culturing the cells according to the invention, more potent and reproducible suppressor cell lines were generated. Furthermore, in view of the art teaching away from using less volume of beads (2 μl vs. 10 to 20 μl), the Examiner has strengthen Applicants contention that the claimed invention is not obvious as discussed more fully below.

For the reasons given, Applicants respectfully request reconsideration and withdrawal of the rejection pursuant to 35 U.S.C. §112, first paragraph.

Rejection of claims 1-5, 7-11, and 28-35 under 35 U.S.C. §103(a)

The Examiner has rejected claims 1-5, 7-11, and 28-35 under 35 U.S.C. § 103(a) as being unpatentable by Schuler *et al.* (US2005/0101012) in view of Baecher-Allan et al. and CD25 Microbead Datasheet (as evidenced by Elkord, 1996 Biocompare Review). Specifically, the Examiner contends that Schuler teaches contacting a sample of $CD4^+$ T cells with anti-CD25 antibody to produce an isolated population of human $CD4^+CD25^+$ T cells and expanding the $CD4^+CD25^+$ T cells with anti-CD3 and anti-CD28 antibodies. Therefore, the Examiner contends that it would have been obvious to one of skill in the art to use the teachings of Schuler with Baecher-Allan to adjust the cell/bead ratio to isolate $CD4^+CD25^+$ T cells and to expand the isolated cells using anti-CD3 and anti-CD28 antibodies.

Applicants respectfully traverse this rejection, and respectfully submit that the combination of art cited by the Examiner does not render the claims obvious under 35 U.S.C. § 103(a) for the following reasons.

According to the U.S. Supreme Court ruling in *Graham v. John Deere*, 383 U.S. 1 (1960), in making a case for obviousness, the Examiner must 1) determine the scope and content of the prior art; 2) ascertain the differences between the prior art and the claims at issue; 3) resolve the level of ordinary skill in the pertinent art; and 4) evaluate evidence of secondary considerations. These principles have been reconfirmed by the Supreme Court in *KSR International Co. v. Teleflex Inc.*, 550 USPQ2d 1385 (2007).

In *KSR Int'l Co.*, the US Supreme Court restated the requirements for a finding of obviousness. Encouraging the application of common knowledge and common sense, the Court took care to guard against hindsight bias and *ex post* reasoning and to distinguish the predictable from the unpredictable arts ("If a person of ordinary skill can implement a predictable variation, §103 likely bars its patentability." [Emphasis added]). Based on the combination of references set forth by the Examiner, Applicant asserts that the rejection of the claims under §103 could only have been made with hindsight bias and *ex post* reasoning.

When applying 35 U.S.C. § 103, the following tenets of patent law must be followed: 1) the claimed invention must be considered as a whole; 2) the references must be considered as a whole; 3) the references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention; and 4) reasonable expectation of success is the standard with which obviousness is determined (MPEP § 2141 II). None of these criteria have been met here.

Claim 1 has been amended to indicate that the isolated cells are further cultured in the presence of a $CD4^+$ feeder layer or $CD4^+$ feeder layer conditioned medium. Nowhere does Schuler teach culturing the cells on a $CD4^+$ feeder layer or $CD4^+$ feeder layer conditioned medium. Accordingly, Schuler does not teach each and every element of claim 1 nor does Schuler render claim 1 obvious.

Nothing in Baecher-Allan et al. and CD25 Microbead Datasheet (as evidenced by Elkord, 1996 Biocompare Review) disclose culturing isolated Tregs in the presence of a $CD4^+$ feeder layer or $CD4^+$ feeder layer conditioned medium. Applicants submit that neither Baecher-Allan nor the CD25 Microbead Datasheet can cure the deficiencies of Schuler. Therefore, because none of the secondary references cited by the Examiner cure the deficiencies of the primary reference, Applicants respectfully submit that the cited references, when taken together, do not render the presently claimed invention obvious.

For the reasons discussed above, the combination of Schuler *et al.* with Baecher-Allan et al. and CD25 Microbead Datasheet does not render claims 1-5, 7-11, and 28-35 obvious under 35 U.S.C. § 103(a) and, therefore, the rejection should be reconsidered and withdrawn.

Summary

Applicant respectfully submits that each rejection of the Examiner to the claims of the present application has been overcome or is now inapplicable, and that the claims are now in condition for allowance. Reconsideration and allowance of these claims is respectfully requested at the earliest possible date.

Respectfully submitted,

BRUCE BLAZAR ET AL.,

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(Date)

By: _____

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